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examining the chemical and biophysical properties of recombinant THB1 (rTHB1) produced in *E. coli* as an apoprotein and recombined with a *b* heme. The goal of the present work was to validate the use of rTHB1 as a surrogate for the native protein. THB1 was partially purified from whole cell extracts of *C. reinhardtii* by chromatographic methods. Enhanced chemiluminescence staining and immunodetection of the protein mixture after native gel electrophoresis showed that THB1 has peroxidase activity. Ultra performance liquid chromatography and mass spectrometry confirmed the association of the polypeptide with a *b* heme and revealed that THB1 is N-terminally acetylated. Nanodrop analysis of the purified extracts returned an optical spectrum consistent with that of recombinant ferric THB1. In addition, the mixture had nitric oxide dioxygenase activity, as observed for rTHB1 [1]. The combined information provides compelling evidence that THB1 uses a *b* heme as cofactor and that the properties of rTHB1 are relevant to THB1 as it is found within the living cell. The observation of a co-translational modification cautions against the sole use of sequence information to derive physiological insight.

[1] Johnson et al. (2014) *Biochemistry* 53:4573

Supported by NSF grant MCB-1330488

1123-Pos Board B74

In Vitro Reconstitution of the Assimilatory Sulfite Reductase from Escherichia Coli

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The central step of sulfur assimilation in Escherichia coli, is catalyzed by the enzyme Sulfite Reductase (SiR). This enzyme reduces sulfite (SO3-2) to sulfide (S-2) in a six electrons transfer reaction. SiR is a multimeric enzymatic complex formed by eight copies of the α-subunit (encoded by the gene cysJ), and four copies of β-subunit (encoded by the gene cysI). NADPH acts as the electron donor that binds to the α-subunit a flavin-binding reductase. Electrons are transfer to the flavin cofactors in the α-subunit to the sulfite that binds to the β-subunit, a metalloprotein. In previous studies the catalytic activity of each subunit has been independently characterized, but it is unclear how they interact in the holoenzyme. Thus, to better study this system we aim to modify each subunit independently and evaluate its effect in the context of the holoenzyme. To achieve that goal is necessary to develop a reconstitution protocol that produce active and stable holoenzyme. Here we show the reconstitution of the SiR complex in vitro, using a His-tagged β-subunit to pull down the α-subunit, from pure protein preparations or cell lysates. The reconstituted SiR is an active enzyme that shows the expected size for a α8β4 complex and it shows the same spectral characteristics as native SiR. Additionally it is highly stable in solution, which has been demonstrated by dynamic light scattering (DLS) experiments.

1124-Pos Board B75

The Functions of the Proteins Encoded by the Cid and Lrg Operons in S. Aureus

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Bacterial programmed cell death (PCD) is an emerging concept with implications in biofilm formation and acquiring of antibiotic resistance. The cid and lrg operons have been shown to be important to PCD in S. aureus and this work focuses on preparing and functionally characterizing proteins encoded by these two operons. The cid operon encodes the CidA, CidB, and CidC proteins, with CidA hypothesized to be a holin-like membrane protein and CidC a membrane-bound pyruvate oxidase. The lrg operon encodes the two membrane proteins LrgA and LrgB, with LrgA hypothesized to be an anti-holin membrane protein. The exact roles of CidB and LrgB remain undetermined to date. Pure preparations of CidA or LrgA were reconstituted into synthetic lipid vesicles that mimic the cellular membrane of S.aureus. A newly developed liposome leakage assay confirms that CidA induces the formation of nanometer membrane pores, while LrgA induces the formation of much smaller membrane pores. Pure recombinant CidC was shown to bind flavin adenine dinucleotide and to exhibit pyruvate oxidase activity in the presence of thiamine pyrophosphate. Ongoing studies include further liposome leakage assays to better define the pores induced by CidA and LrgA, as well as any influence exterted by the CidB and LrgB proteins. The interaction between Cid/Lrg membrane proteins is also investigated by isothermal titration calorimetry. This research will elucidate the in vivo functions of the Cid/Lrg proteins and shed light on bacterial programmed cell death.

1125-Pos Board B76

Effects of Siroheme Occupancy in Escherichia Coli Assimilatory Sulfite Reductase Hemoprotein

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Sulfur reduction is a fundamental biological process in most bacteria and plants. As a model system we study the *Escherichia coli* assimilatory sulfite reductase (aSiR), an ~800kD complex that catalyzes the concerted six-electron reduction of sulfite (SO_3^{-2}) to biologically useful sulfide (S^{-2}). The complex consists of alpha and beta subunits, the flavoprotein and hemoprotein respectively. The active site lies in the hemoprotein subunit (SiRHP) that contains a siroheme cofactor coupled to a 4Fe-4S cluster used to shuttle electrons to the bound substrate.

To investigate the structural importance of siroheme in SiRHP, we removed the cofactor by both mutagenic and auxotrophic methods. Electrophoretic mobility shift assays, circular dichroism, and dynamic light scattering were used to characterize changes in SiRHP lacking siroheme (apo SIRHP). In addition, the occupancy of the 4Fe-4S cluster was spectroscopically probed using an Ellman's reagent test. Apo SiRHP was found to exist as a tetramer instead of a monomer and no longer contained the 4Fe-4S cluster. Additionally, the apo SiRHP tetramer formed by the absence of siroheme was unable to bind the flavoprotein subunit required for functional aSiR assembly. These results suggest a possible mechanism for removing non-functional SiRHP subunits from the assembly pool within the cell.

1126-Pos Board B77

Ligand-Binding and Substrate Turnover of a Heme Peroxidase from the Diatom Thalassiosira Pseudonana

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Iron is sparingly soluble in water; therefore, iron-containing enzymes in marine organisms such as diatoms must have critical physiological roles to justify the use of such a precious resource. We have identified a putative heme peroxidase of unknown function, Tp 21683, from the published genome of the model diatom Thalassiosira pseudonana. Conserved domain analysis suggests Tp 21683 consists of an N-terminal kringle domain, likely involved in ligand binding and localization, and a C-terminal b-type heme peroxidase domain. To dissect the function of the two domains of Tp 21683, we have optimized recombinant expression in Escherichia coli and subsequent refolding and purification of the isolated kringle and peroxidase domains. We report preliminary studies to identify the ligand-binding and peroxidase turnover properties of Tp 21683 toward a better understanding of the physiological role of peroxidases in diatoms. We show by intrinsic tryptophan fluorescence that unlike canonical kringle domains from human plasminogen, the kringle domain of Tp 21683 does not show measurable binding to lysine. However, the kringle domain appears to bind to the oleic acid with a Kd of approximately 1 μM. The peroxidase domain is able to turn over hydrogen peroxide using both the nonphysiological electron donor ABTS and the aromatic compound guaiacol with kcat of approximately 40 min-1 and 4 min-1, respectively. These data are the first analysis of a recombinant peroxidase from a diatom, and provide a basis for further investigation of the range of possible ligands and chemical reactions catalyzed by Tp 21683.

1127-Pos Board B78

Single Molecule Activity Measurements of Cytochrome P450 Oxidoreductase Reveal the Existence of Two Discrete Functional States

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Electron transfer between membrane spanning oxidoreductases crucially controls vital metabolic processes. An important member of the oxidoreductase superfamily, Cytochrome P450 Oxidoreductase is the canonical membrane spanning activator of all microsomal (>50) P450 enzymes and involved in steroid hormone biosynthesis, xenobiotic drug catabolism(1). The mechanism underlying POR functional plasticity, its ability to select one out of a plethora of